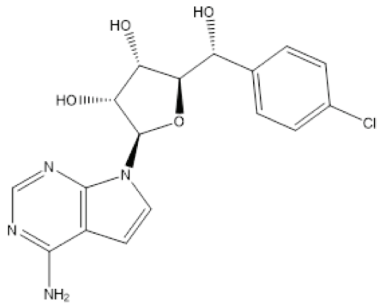
PRMT5 inhibition modulates E2F1 methylation and gene regulatory networks leading to therapeutic efficacy in JAK2V617F mutant MPN

Data Supplement

Supplementary Figures

FIGURE S1

A



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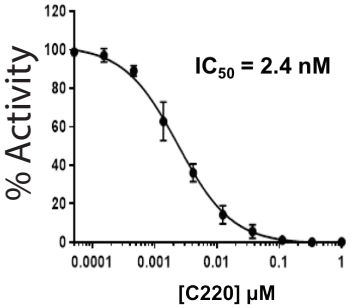

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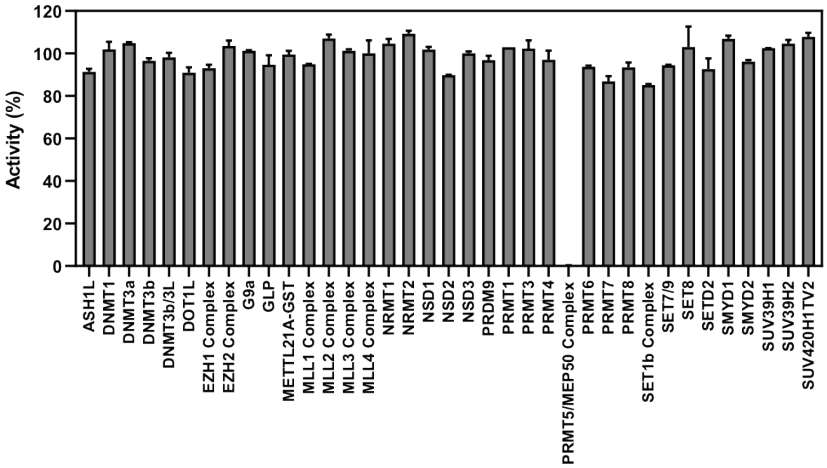


Figure S1: Structure and biochemical characterization of C220.

(A) Structure of C220.

(B) Enzyme inhibition assay of PRMT5/MEP50 complex using histone H4 based peptide. IC50 was determined to be 2.4 nM (Hill Slope of 1.12).

(C) C220 was tested at a concentration of 10 μM against a panel of 38 methyltransferases and it was found to be highly selective at inhibiting activity of the PRMT5/MEP50 complex as compared to other related family members.

FIGURE S2

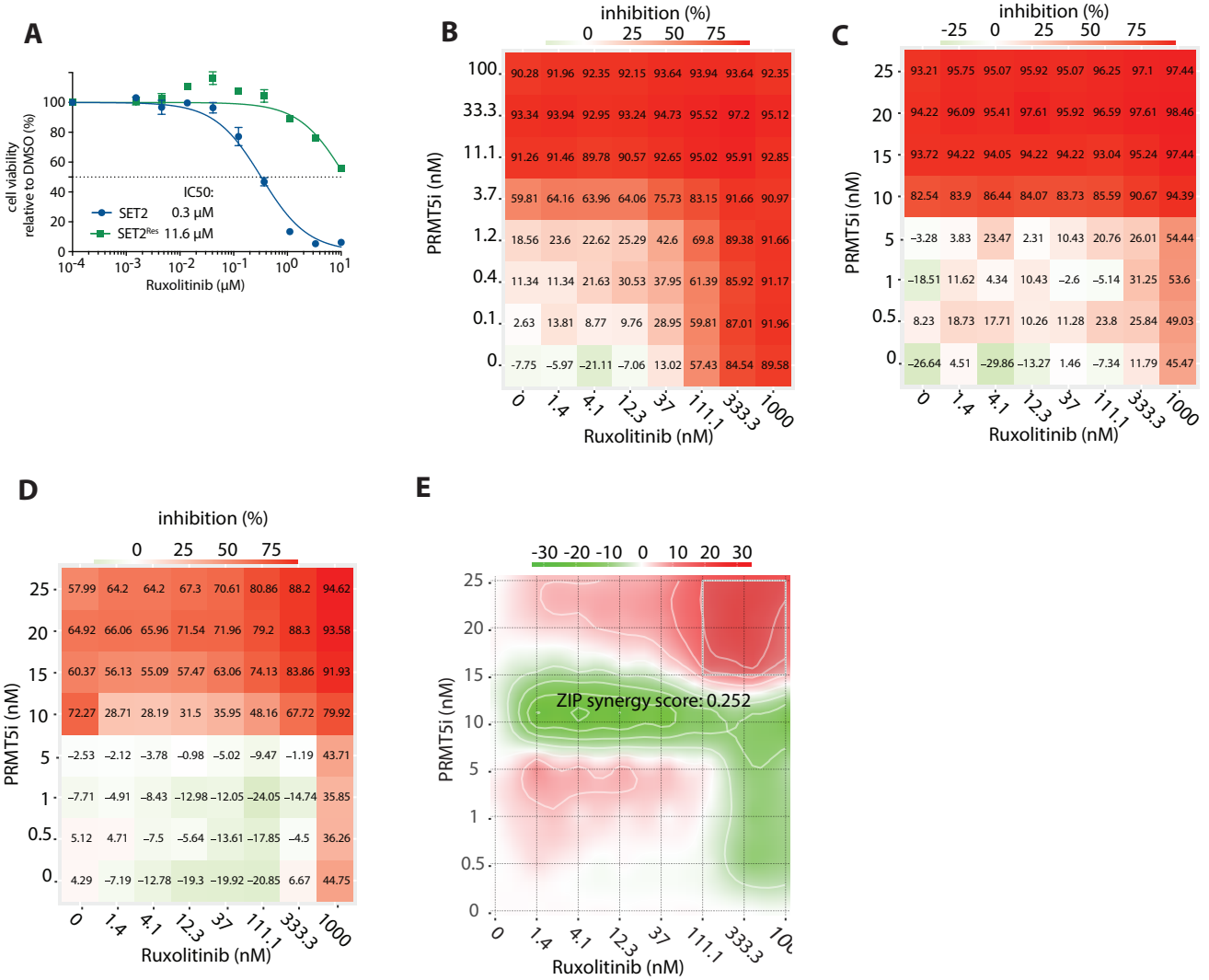

A
cell viability relative to DMSO (%)
IC50:
SET2 0.3 µM
SET2Res 11.6 µM
Ruxolitinib (µM)
B
inhibition (%)
PRMT5i (nM)
Ruxolitinib (nM)
C
inhibition (%)
PRMT5i (nM)
Ruxolitinib (nM)
D
inhibition (%)
PRMT5i (nM)
Ruxolitinib (nM)
E
PRMT5i (nM)
ZIP synergy score: 0.252
Ruxolitinib (nM)

Figure S2: Inhibition of PRMT5 by C220 shows efficacy in JAK2V617F transduced Ba/F3 cells and JAK2V617 heterozygous SET2 *in vitro*

(A) Proliferation with increasing concentration of Ruxolitinib (µM) relative to proliferation in the presence of DMSO control is depicted for SET2 and Ruxolitinib-resistent SET2 cells ($SET2^{Res}$). IC50 values for Ruxolitinib are indicated on the right. Data of indicated as mean ± SEM.

(B) Visualization of the dose-response matrix and the plots of phenotypic responses for the single C220 and ruxolitinib treatment in SET2 cells treated for 6 days.

(C) Visualization of the dose-response matrix and the plots of phenotypic responses for the single C220 and ruxolitinib treatment in Ba/F3 cells transduced with JAK2V617F treated for 7 days.

(D) Visualization of the dose-response matrix and the plots of phenotypic responses for the single C220 and ruxolitinib treatment in Ba/F3 cells transduced with JAK2-wildtype treated for 7 days.

(E) Raw dose-response matrix data derived from proliferation synergy assay for the combination of PRMT5 inhibition by C220 and JAK1/2 inhibition by ruxolitinib in JAK2-wildtype transduced Ba/F3 cells is visualized as a heatmap.

FIGURE S3

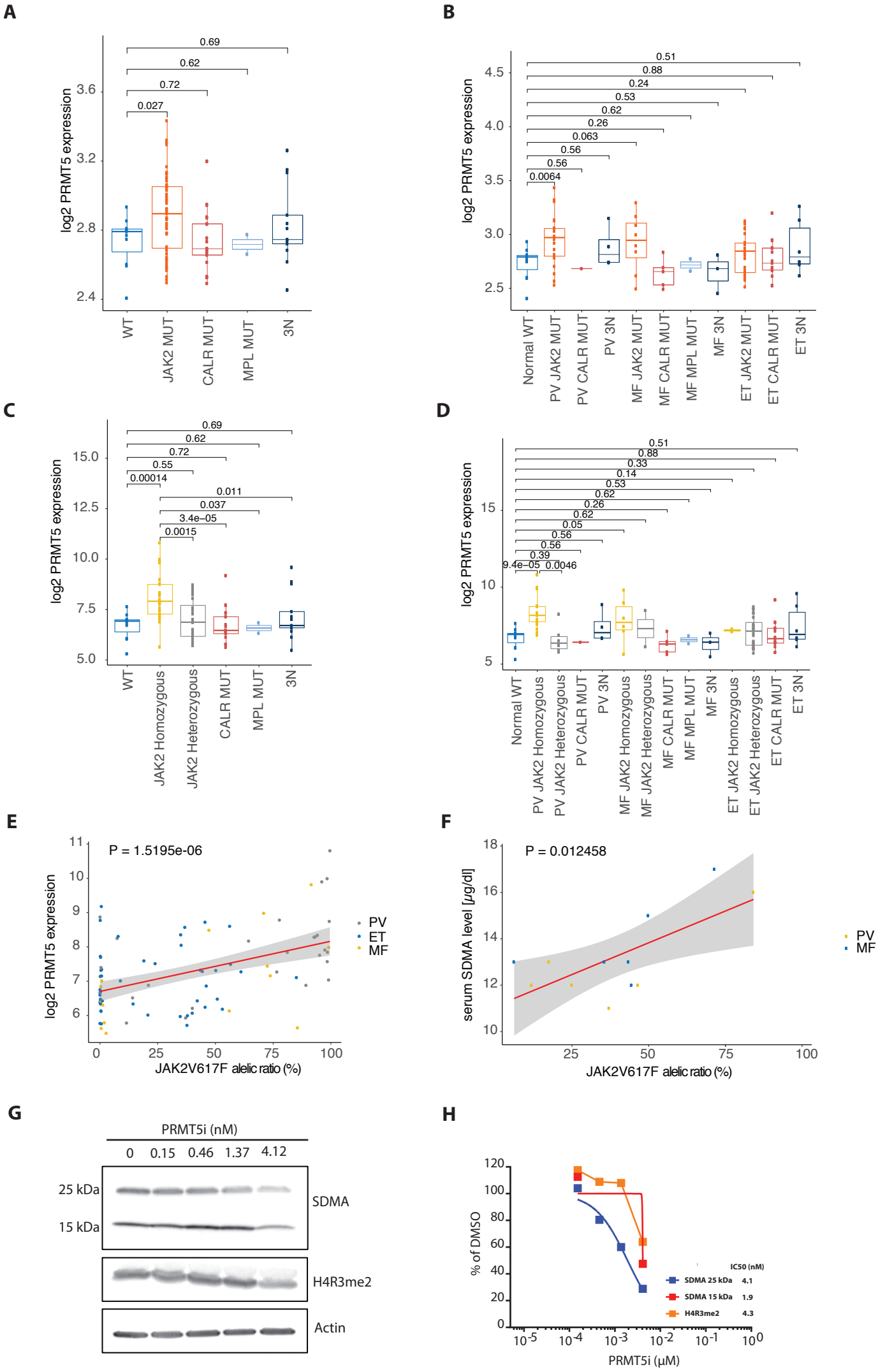

A
log2 PRMT5 expression
WT
JAK2 MUT
CALR MUT
MPL MUT
3N
B
log2 PRMT5 expression
Normal WT
PV JAK2 MUT
PV CALR MUT
PV 3N
MF JAK2 MUT
MF CALR MUT
MF MPL MUT
MF 3N
ET JAK2 MUT
ET CALR MUT
ET 3N
C
log2 PRMT5 expression
WT
JAK2 Homozygous
JAK2 Heterozygous
CALR MUT
MPL MUT
3N
D
log2 PRMT5 expression
Normal WT
PV JAK2 Homozygous
PV JAK2 Heterozygous
PV CALR MUT
PV 3N
MF JAK2 Homozygous
MF JAK2 Heterozygous
MF CALR MUT
MF MPL MUT
MF 3N
ET JAK2 Homozygous
ET JAK2 Heterozygous
ET CALR MUT
ET 3N
E
P = 1.5195e-06
log2 PRMT5 expression
JAK2V617F alelic ratio (%)
PV
ET
MF
F
P = 0.012458
serum SDMA level [μg/dl]
JAK2V617F alelic ratio (%)
PV
MF
G
PRMT5i (nM)
0
0.15
0.46
1.37
4.12
25 kDa
15 kDa
SDMA
H4R3me2
Actin
H
% of DMSO
PRMT5i (μM)
IC50 (nM)
SDMA 25 kDa 4.1
SDMA 15 kDa 1.9
H4R3me2 4.3

Figure S3: PRMT5 expression levels and serum SDMA levels in MPN subgroups and efficacy of PRMT5 inhibition by C220 in reducing SDMA in levels in patient derived CD34+ cells *ex vivo*

(A) PRMT5 expression in peripheral blood granulocytes in mutational subgroups defined by the presence or absence of JAK2, CALR and MPL mutations or triple negative cases (3N) in all MPN patients. Analysis of publicly available data(10).
(B) PRMT5 expression in peripheral blood granulocytes in mutational subgroups defined by the presence or absence of JAK2, CALR and MPL mutations or triple negative cases (3N) in PV, ET and MF patients. Analysis of publicly available data(10).
(C) PRMT5 expression in peripheral blood granulocytes in mutational subgroups defined by the presence or absence of JAK2 homozygous or JAK2 heterozygous mutations, CALR and MPL mutations or triple negative cases (3N) in all MPN patients. JAK2V617F allele burden was measured by TaqMan and a value of >50 was defined as a homozygous JAK2V617F mutation. Analysis of publicly available data(10).
(D) PRMT5 expression in peripheral blood granulocytes in mutational subgroups defined by the presence or absence of JAK2 homozygous or JAK2 heterozygous mutations, CALR and MPL mutations or triple negative cases (3N) in in PV, ET and MF patients. JAK2V617F allele burden was measured by TaqMan and a value of >50 was defined as a homozygous JAK2V617F mutation. Analysis of publicly available data(10).
(E) Correlation between PRMT5 expression in peripheral blood granulocytes and JAK2V617 allele burden in ET (n=47), PV (n=28) and MF (n=18) patients. JAK2V617F allele burden was measured by TaqMan. Analysis of publicly available data(10).
(F) Correlation between SDMA serum levels and JAK2V617 allele burden JAK2V617F+ PV (n=6) and PMF (n=6) patients. JAK2V617F allele burden was measured by TaqMan(10).
(G) Western Blot assessment of SDMA at 15kDa and 25kDa and H4R3me2 in cells derived from an ex vivo treatment of CD34+ cells from a JAK2V617F PV patient using different PRMT5 inhibitor concentrations and DMSO.
(H) Graphical presentation of measuring symmetric arginine dimethylation using either H4R3me2 or SDMA expression (at 15 kD and 25 kDa) in Western Blot from an ex vivo treatment of CD34+ cells from a JAK2V617F PV patient using different PRMT5 inhibitor concentrations and DMSO.

FIGURE S4

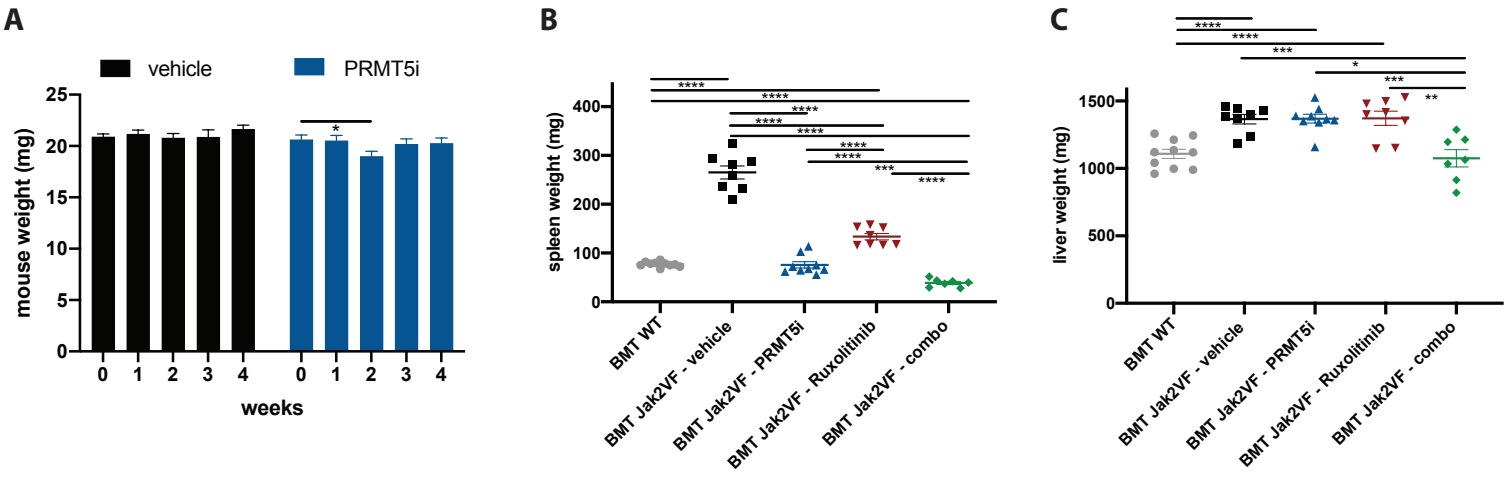

A
vehicle
PRMT5i
mouse weight (mg)
25
20
15
10
5
0
0 1 2 3 4 0 1 2 3 4
weeks
*
B
spleen weight (mg)
400
300
200
100
0
****
****
****
****
****
****
***
****
BMT WT
BMT Jak2VF - vehicle
BMT Jak2VF - PRMT5i
BMT Jak2VF - Ruxolitinib
BMT Jak2VF - combo
C
liver weight (mg)
1500
1000
500
0
****
****
***
*
***
**
BMT WT
BMT Jak2VF - vehicle
BMT Jak2VF - PRMT5i
BMT Jak2VF - Ruxolitinib
BMT Jak2VF - combo

Figure S4: Assessment of a potential toxicity of single and combined PRMT5i/ruxolitinib treatment in the JAK2V617F mouse model

A) Course of mouse body weights of JAK2V617F chimeric transplanted B6 mice treated with PRMT5i (15 mg/kg) or vehicle for 4 weeks. Data are indicated as mean ± SEM.
B) Spleen weights of JAK2V617F chimeric transplanted mice at 4 weeks of treatment with C220 at 12.5 mg/kg, ruxolitinib 60 mg/kg, combination of both or vehicle compared to sex- and age-matched untreated wildtype B6 transplant recipient control mice. Data are represented as mean +/- SEM (****p<0.0001, ***p<0.001).
C) Liver weights of JAK2V617F chimeric transplanted mice at 4 weeks of treatment with C220 at 12.5 mg/kg, ruxolitinib 60 mg/kg, combination of both or vehicle compared to sex- and age-matched untreated wildtype B6 transplant recipient control mice. Data are represented as mean +/- SEM (****p<0.0001, ***p<0.001, **p<0.01).

FIGURE S5

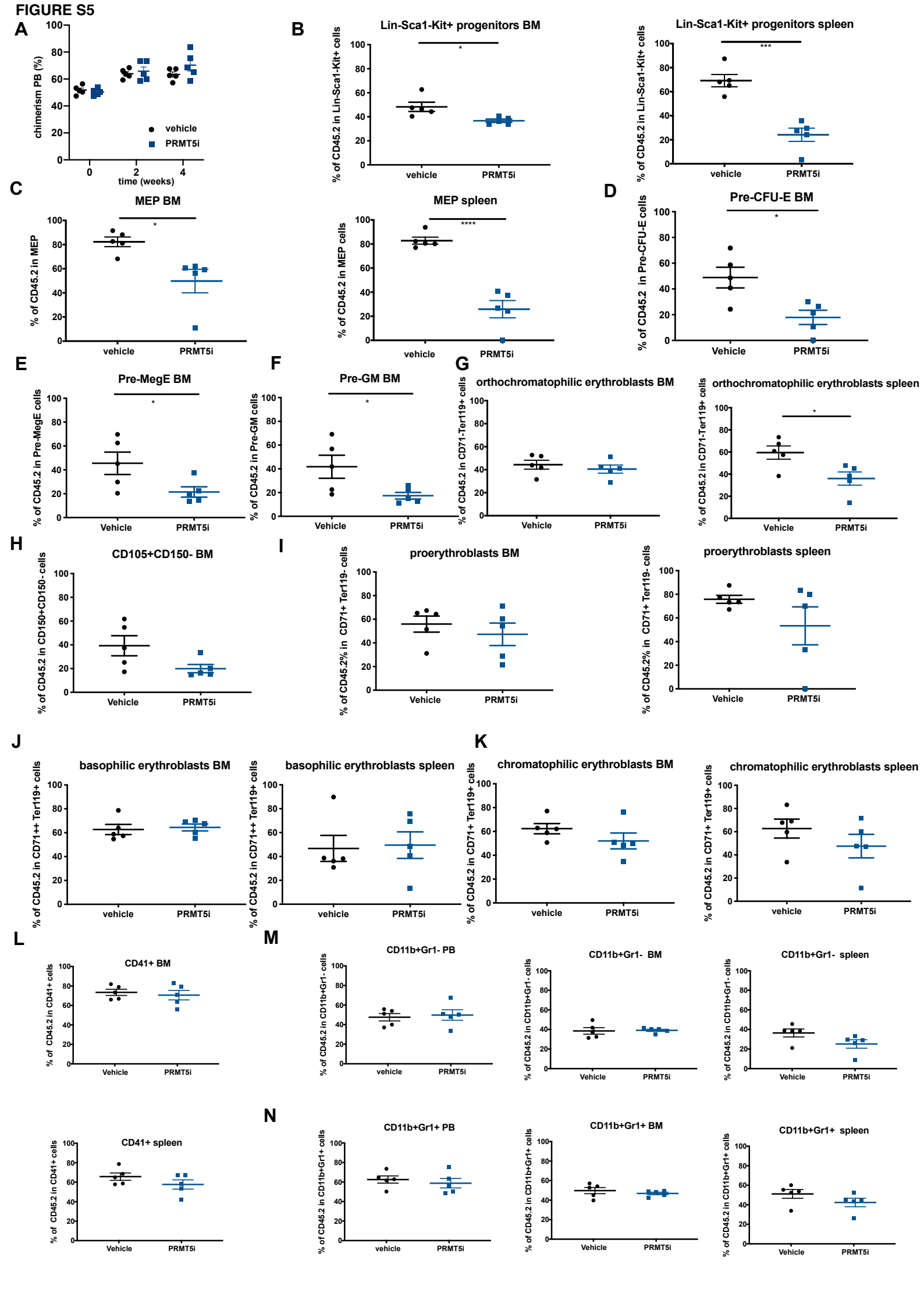

A
chimerism PB (%)
vehicle
PRMT5i
time (weeks)
B
Lin-Sca1-Kit+ progenitors BM
% of CD45.2 in Lin-Sca1-Kit+ cells
Lin-Sca1-Kit+ progenitors spleen
C
MEP BM
% of CD45.2 in MEP
MEP spleen
% of CD45.2 in MEP cells
D
Pre-CFU-E BM
% of CD45.2 in Pre-CFU-E cells
E
Pre-MegE BM
% of CD45.2 in Pre-MegE cells
F
Pre-GM BM
% of CD45.2 in Pre-GM cells
G
orthochromatophilic erythroblasts BM
% of CD45.2 in CD71-Ter119+ cells
orthochromatophilic erythroblasts spleen
H
CD105+CD150- BM
% of CD45.2 in CD150+CD150- cells
I
proerythroblasts BM
% of CD45.2% in CD71+ Ter119- cells
proerythroblasts spleen
J
basophilic erythroblasts BM
% of CD45.2 in CD71++ Ter119+ cells
basophilic erythroblasts spleen
K
chromatophilic erythroblasts BM
chromatophilic erythroblasts spleen
L
CD41+ BM
% of CD45.2 in CD41+ cells
CD41+ spleen
M
CD11b+Gr1- PB
% of CD45.2 in CD11b+Gr1- cells
CD11b+Gr1- BM
CD11b+Gr1- spleen
N
CD11b+Gr1+ PB
% of CD45.2 in CD11b+Gr1+ cells
CD11b+Gr1+ BM
CD11b+Gr1+ spleen
vehicle
PRMT5i
Vehicle

Figure S5: Detailed CD45.2 (JAK2V617F) versus CD45.1 (wildtype) subgroup analysis derived from chimeric JAK2V617F/WT transplants treated with C220

A) Peripheral blood chimerism is assessed by flow cytometry at randomization and after 2 and 4 weeks of treatment with 12.5 mg/kg C220 in the CD45.1/CD45.2 competitive conditional knock-in model of PV. Data are represented as mean +/- SEM.

B) % of CD45.2+ (=JAK2V617F+) Lin-Sca1-cKit+ myeloid progenitors in the BM and spleen after 4 weeks of treatment with 12.5 mg/kg C220 in the CD45.1/CD45.2 competitive conditional knock-in model of PV. Data are represented as mean +/- SEM (***p<0.001, *p<0.05).

C) % of CD45.2+ (=JAK2V617F+) MEP in the BM and spleen after 4 weeks of treatment with 12.5 mg/kg C220 in the CD45.1/CD45.2 competitive conditional knock-in model of PV. Data are represented as mean +/- SEM (****p<0.0001, *p<0.05).

D) % of CD45.2+ (=JAK2V617F+) Lin-cKit^{high}CD41-FcgR-CD150+CD105+ committed erythroid progenitors (Pre-CFU-E) in the BM after 4 weeks of treatment with 12.5 mg/kg C220 in the CD45.1/CD45.2 competitive conditional knock-in model of PV. Data are represented as mean +/- SEM (*p<0.05).

E) % of CD45.2+ (=JAK2V617F+) Lin-cKit^{high}CD41-FcgR-CD150+CD105- bipotential megakaryocyte-erythroid progenitors (Pre-Meg-E) in the BM after 4 weeks of treatment with 12.5 mg/kg C220 in the CD45.1/CD45.2 competitive conditional knock-in model of PV. Data are represented as mean +/- SEM (*p<0.05).

F) % of CD45.2+ (=JAK2V617F+) Lin-cKit^{high}CD41-FcgR-CD150-CD105- pre-granulocyte macrophage progenitors (Pre-GM) in the BM after 4 weeks of treatment with 12.5 mg/kg C220 in the CD45.1/CD45.2 competitive conditional knock-in model of PV. Data are represented as mean +/- SEM (*p<0.05).

G) % of CD45.2+ (=JAK2V617F+) Ter119^{high}CD71^{low} orthochromatophilic erythroblasts in the BM and spleen after 4 weeks of treatment with 12.5 mg/kg C220 in the CD45.1/CD45.2 competitive conditional knock-in model of PV. Data are represented as mean +/- SEM (*p<0.05).

H) % of CD45.2+ (=JAK2V617F+) Lin-cKit^{high}CD41-FcgR-CD150-CD105+ progenitors in the BM after 4 weeks of treatment with 12.5 mg/kg C220 in the CD45.1/CD45.2 competitive conditional knock-in model of PV. Data are represented as mean +/- SEM.

I) % of CD45.2+ (=JAK2V617F+) Ter119^{med}CD71^{high} proerythroblasts in the BM and spleen after 4 weeks of treatment with 12.5 mg/kg C220 in the CD45.1/CD45.2 competitive conditional knock-in model of PV. Data are represented as mean +/- SEM.

J) % of CD45.2+ (=JAK2V617F+) Ter119^{high}CD71^{high} basophilic erythroblasts in the BM and spleen after 4 weeks of treatment with 12.5 mg/kg C220 in the CD45.1/CD45.2 competitive conditional knock-in model of PV. Data are represented as mean +/- SEM.

K) % of CD45.2+ (=JAK2V617F+) Ter119^{high}CD71^{med} chromatophilic erythroblasts in the BM and spleen after 4 weeks of treatment with 12.5 mg/kg C220 in the CD45.1/CD45.2 competitive conditional knock-in model of PV. Data are represented as mean +/- SEM.

L) % of CD45.2+ (=JAK2V617F+) CD41+ cells in the BM and spleen after 4 weeks of treatment with 12.5 mg/kg C220 in the CD45.1/CD45.2 competitive conditional knock-in model of PV. Data are represented as mean +/- SEM.

M) % of CD45.2+ (=JAK2V617F+) CD11b+Gr1- cells in the PB, BM and spleen after 4 weeks of treatment with 12.5 mg/kg C220 in the CD45.1/CD45.2 competitive conditional knock-in model of PV. Data are represented as mean +/- SEM.

N) % of CD45.2+ (=JAK2V617F+) CD11b+Gr1+ cells in the PB, BM and spleen after 4 weeks of treatment with 12.5 mg/kg C220 in the CD45.1/CD45.2 competitive conditional knock-in model of PV. Data are represented as mean +/- SEM.

FIGURE S6

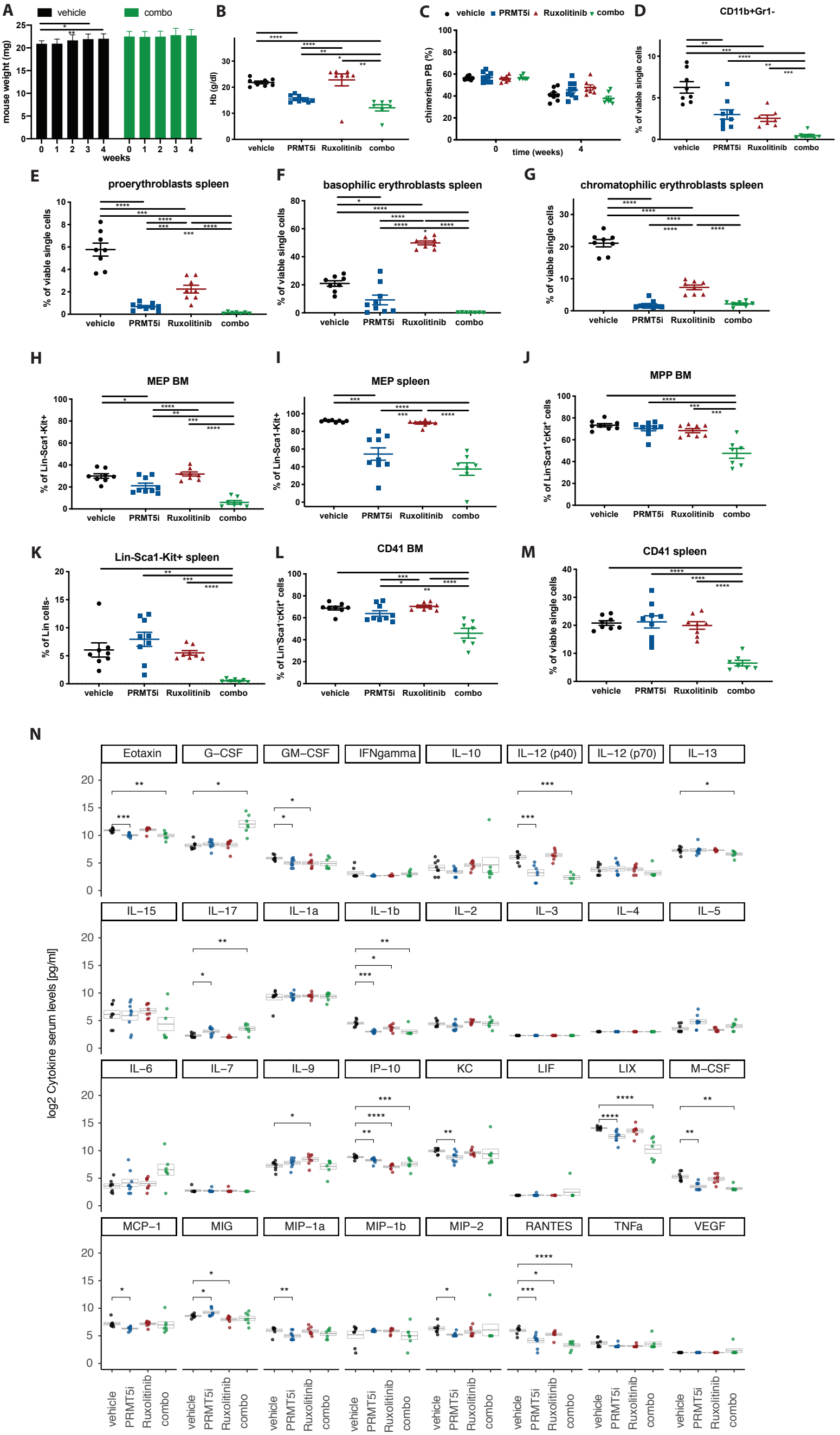

A
vehicle
combo
mouse weight (mg)
weeks
B
Hb (g/dl)
vehicle
PRMT5i
Ruxolitinib
combo
C
vehicle PRMT5i Ruxolitinib combo
chimerism PB (%)
time (weeks)
D
CD11b+Gr1-
% of viable single cells
E
proerythroblasts spleen
F
basophilic erythroblasts spleen
G
chromatophilic erythroblasts spleen
H
MEP BM
% of Lin-Sca1-Kit+
I
MEP spleen
J
MPP BM
% of Lin-Sca1+cKit+ cells
K
Lin-Sca1-Kit+ spleen
% of Lin cells-
L
CD41 BM
% of Lin-Sca1-cKit+ cells
M
CD41 spleen
N
Eotaxin
G−CSF
GM−CSF
IFNgamma
IL−10
IL−12 (p40)
IL−12 (p70)
IL−13
IL−15
IL−17
IL−1a
IL−1b
IL−2
IL−3
IL−4
IL−5
IL−6
IL−7
IL−9
IP−10
KC
LIF
LIX
M−CSF
MCP−1
MIG
MIP−1a
MIP−1b
MIP−2
RANTES
TNFa
VEGF
log2 Cytokine serum levels [pg/ml]
O
Actin
SDMA
G1-1 G1-2 G1-3 G1-4 G3-1 G3-2 G3-3 G3-4 G4-1 G4-2 G4-3 G6-1 G6-4 G6-5 G6-6 G3-8
SDMA normalized to Actin

Figure S6: Dual PRMT5 and JAK1/2 inhibition is superior to C220 or ruxolitinib monotherapy in the conditional JAK2V617F knock-in model of PV

A) Course of mouse body weights of JAK2V617F chimeric transplanted B6 mice treated with combined PRMT5i (10 mg/kg)/ruxolitinib (60 mg/kg) or vehicle for 4 weeks. Data are indicated as mean ± SEM (**p<0.01, (*p<0.05).

B) Hemoglobin levels at 4 weeks of treatment with C220 at 12.5 mg/kg, Ruxolitinib 60 mg/kg or combination of both versus vehicle. Data are represented as mean +/- SEM (****p<0.0001, **p<0.01, *p<0.05).

C) Peripheral blood chimerism is assessed by flow cytometry at randomization and after 4 weeks of treatment with C220 at 12.5 mg/kg, Ruxolitinib 60 mg/kg or combination of both versus vehicle. Data are represented as mean +/- SEM.

D) CD11b+Gr1- cells in the peripheral blood at 4 weeks of treatment with C220 at 12.5 mg/kg, Ruxolitinib 60 mg/kg or combination of both versus vehicle. Data are represented as mean +/- SEM (****p<0.0001, ***p<0.001, **p<0.01).

E) The $Ter119^{med}CD71^{high}$ proerythroblast population in C220 (12.5 mg/kg), Ruxolitinib (60 mg/kg), combination of both or vehicle treated animals is illustrated as means +/- SEM (****p<0.0001, ***p<0.001).

F) The $Ter119^{high}CD71^{high}$ basophilic erythroblast populations in C220 (12.5 mg/kg), Ruxolitinib (60 mg/kg), combination of both or vehicle treated animals are illustrated as means +/- SEM (****p<0.0001, *p<0.05).

G) The $Ter119^{high}CD71^{med}$ chromatophilic erythroblast populations in C220 (12.5 mg/kg), Ruxolitinib (60 mg/kg), combination of both or vehicle treated animals are illustrated as means +/- SEM (****p<0.0001).

H) MEP in the bone marrow at 4 weeks of treatment with C220 at 12.5 mg/kg, Ruxolitinib 60 mg/kg or combination of both versus vehicle. Data are represented as mean +/- SEM (****p<0.0001, ***p<0.001, **p<0.01, *p<0.05).

I) MEP in the spleen at 4 weeks of treatment with C220 at 12.5 mg/kg, Ruxolitinib 60 mg/kg or combination of both versus vehicle. Data are represented as mean +/- SEM (****p<0.0001, ***p<0.001).

J) Lin-Sca1+cKit+CD48-CD150- multipotent myeloid progenitors (MPP) in the bone marrow at 4 weeks of treatment with C220 at 12.5 mg/kg, Ruxolitinib 60 mg/kg or combination of both versus vehicle. Data are represented as mean +/- SEM (****p<0.0001, ***p<0.001).

K) Lin-Sca1-cKit+ myeloid progenitors in the spleen at 4 weeks of treatment with C220 at 12.5 mg/kg, Ruxolitinib 60 mg/kg or combination of both versus vehicle. Data are represented as mean +/- SEM (****p<0.0001, ***p<0.001, **p<0.01).

L) CD41 cells in the bone marrow at 4 weeks of treatment with C220 at 12.5 mg/kg, Ruxolitinib 60 mg/kg or combination of both versus vehicle. Data are represented as mean +/- SEM (****p<0.0001, ***p<0.001, **p<0.01, 0<0.05).

M) CD41 cells in the spleen at 4 weeks of treatment with C220 at 12.5 mg/kg, Ruxolitinib 60 mg/kg or combination of both versus vehicle. Data are represented as mean +/- SEM (****p<0.0001).

N) Serum cytokine levels (log2) of all 32 cytokines assessed by MCYT MAG- 70K- PX32 (EMD Millipore) at 4 weeks of treatment with C220 at 12.5 mg/kg, Ruxolitinib 60 mg/kg or combination of both versus vehicle are illustrated. Data are represented as median with 25^{th} and 75^{th} percentiles. p-values between each single and the combination therapy versus vehicle are plotted (****p<0.0001, ***p<0.001, **p<0.01, 0<0.05).

O) SDMA expression in tumors derived from SET2 cell derived xenografts after oral treatment with C220 at 15 mg/kg, Ruxolitinib or combination therapy or vehicle for 2 weeks is assessed by Western blot and depicted normalized to actin. Data are represented as mean +/- SEM (***p<0.001, **p<0.01).

FIGURE S7

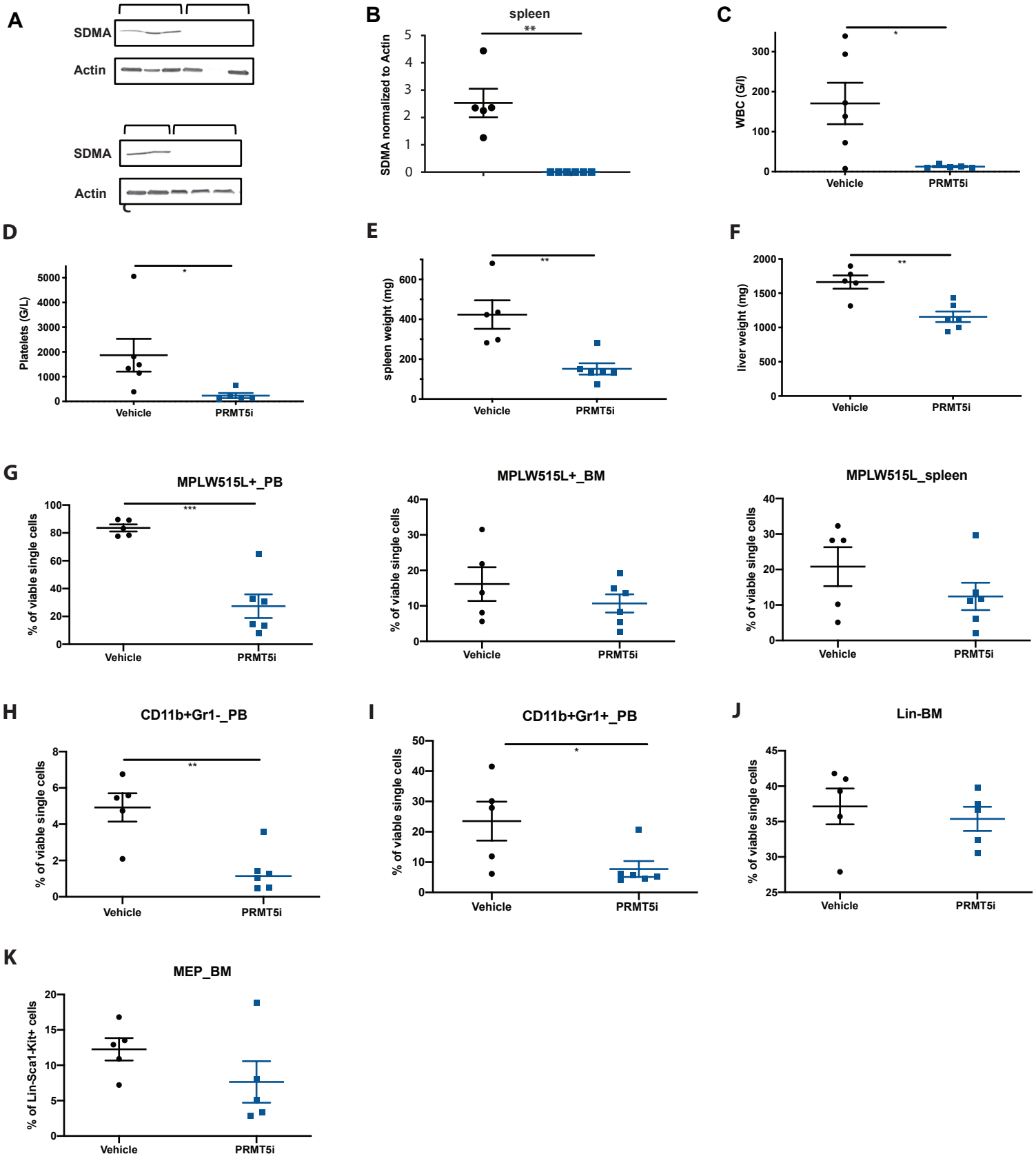

A
SDMA
Actin
SDMA
Actin
B
spleen
**
SDMA normalized to Actin
C
*
WBC (G/l)
Vehicle
PRMT5i
D
*
Platelets (G/L)
Vehicle
PRMT5i
E
**
spleen weight (mg)
Vehicle
PRMT5i
F
**
liver weight (mg)
Vehicle
PRMT5i
G
MPLW515L+_PB
***
% of viable single cells
Vehicle
PRMT5i
MPLW515L+_BM
% of viable single cells
Vehicle
PRMT5i
MPLW515L_spleen
% of viable single cells
Vehicle
PRMT5i
H
CD11b+Gr1-_PB
**
% of viable single cells
Vehicle
PRMT5i
I
CD11b+Gr1+_PB
*
% of viable single cells
Vehicle
PRMT5i
J
Lin-BM
% of viable single cells
Vehicle
PRMT5i
K
MEP_BM
% of Lin-Sca1-Kit+ cells
Vehicle
PRMT5i

Figure S7: PRMT5 inhibition shows increased efficacy in the *MPL*W515L model

A) SDMA expression in spleen is assessed at 3 weeks of treatment with C220 at 15 mg/kg versus vehicle.
B) SDMA expression in spleen normalized to actin at 3 weeks of treatment with C220 at 15 mg/kg versus vehicle (**p<0.01).
C) WBC at 3 weeks of treatment with C220 at 15 mg/kg versus vehicle. Data are represented as mean +/- SEM (*p<0.05).
D) Platelet counts at 3 weeks of treatment with C220 at 15 mg/kg versus vehicle. Data are represented as mean +/- SEM (*p<0.05).
E) Spleen weights at 3 weeks of treatment with C220 at 15 mg/kg versus vehicle. Data are represented as mean +/- SEM (**p<0.01 versus vehicle group).
F) Liver weights at 3 weeks of treatment with C220 at 15 mg/kg versus vehicle. Data are represented as mean +/- SEM (**p<0.01 versus vehicle group).
G) MPLW515L+ (GFP+) cells in the peripheral blood, bone marrow and spleen at 3 weeks of treatment with C220 at 15 mg/kg versus vehicle. Data are represented as mean +/- SEM (***p<0.001 versus vehicle group).
H) CD11b+Gr1- cells in the peripheral blood at 3 weeks of treatment with C220 at 15 mg/kg, versus vehicle. Data are represented as mean +/- SEM (**p<0.01).
I) CD11b+Gr1+ cells in the peripheral blood at 3 weeks of treatment with C220 at 15 mg/kg, versus vehicle. Data are represented as mean +/- SEM (*p<0.05).
J) Lin- cells in the bone marrow at 3 weeks of treatment with C220 at 15 mg/kg, versus vehicle. Data are represented as mean +/- SEM.
K) MEP in the bone marrow at 3 weeks of treatment with C220 at 15 mg/kg, versus vehicle. Data are represented as mean +/- SEM.

FIGURE S8

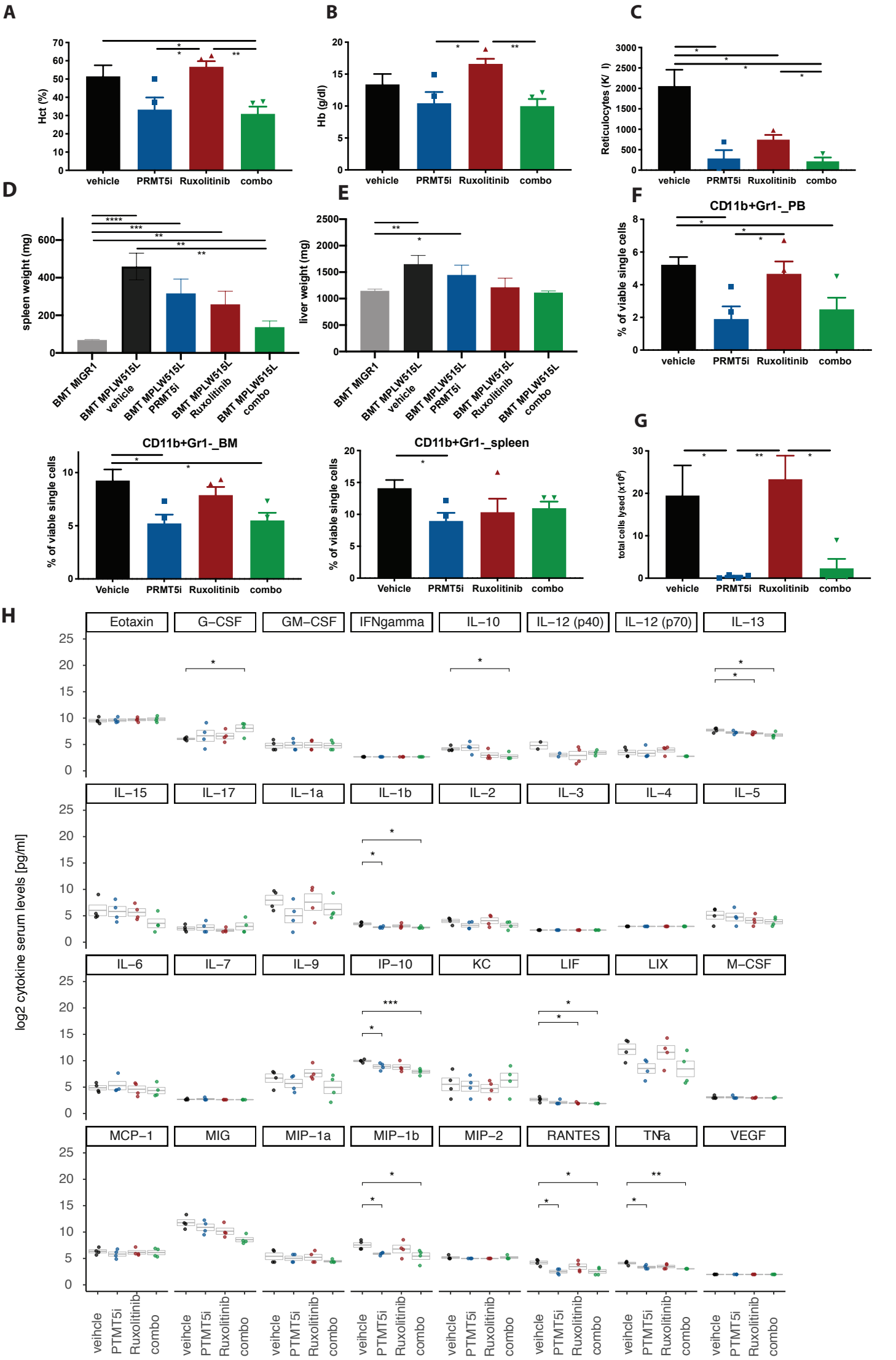

A
Hct (%)
vehicle
PRMT5i
Ruxolitinib
combo
B
Hb (g/dl)
C
Reticulocytes (K/ l)
D
spleen weight (mg)
BMT MIGR1
BMT MPLW515L vehicle
BMT MPLW515L PRMT5i
BMT MPLW515L Ruxolitinib
BMT MPLW515L combo
E
liver weight (mg)
F
CD11b+Gr1-_PB
% of viable single cells
CD11b+Gr1-_BM
Vehicle
CD11b+Gr1-_spleen
G
total cells lysed (x10^6)
H
Eotaxin
G–CSF
GM–CSF
IFNgamma
IL–10
IL–12 (p40)
IL–12 (p70)
IL–13
IL–15
IL–17
IL–1a
IL–1b
IL–2
IL–3
IL–4
IL–5
IL–6
IL–7
IL–9
IP–10
KC
LIF
LIX
M–CSF
MCP–1
MIG
MIP–1a
MIP–1b
MIP–2
RANTES
TNFa
VEGF
log2 cytokine serum levels [pg/ml]
veihcle
PTMT5i
Ruxolitinib
combo

Figure S8: Dual PRMT5 and JAK1/2 inhibition shows increased efficacy in the *MPL*W515L model

A) Hematocrit at 3 weeks of treatment with C220 at 10 mg/kg, Ruxolitinib 60 mg/kg or combination of both versus vehicle. Data are represented as mean +/- SEM (**p<0.01, *p<0.05).
B) Hemoglobin levels at 3 weeks of treatment with C220 at 10 mg/kg, Ruxolitinib 60 mg/kg or combination of both versus vehicle. Data are represented as mean +/- SEM (**p<0.01, *p<0.05).
C) Reticulocyte counts at 3 weeks of treatment with C220 at 10 mg/kg, Ruxolitinib 60 mg/kg or combination of both versus vehicle. Data are represented as mean +/- SEM (*p<0.05).
D) Spleen weights of MSCV-IRES-GFP_MPLW515L transplanted BALB/c mice at 3 weeks of treatment with C220 at 10 mg/kg, ruxolitinib 60 mg/kg, combination of both or vehicle compared to sex- and age-matched untreated MSCV-IRES-GFP_empty vector transplanted BALB/c control mice. Data are represented as mean +/- SEM (****p<0.0001, ***p<0.001, **p<0.01).
E) Liver weights of of MSCV-IRES-GFP_MPLW515L transplanted BALB/c mice at 3 weeks of treatment with C220 at 10 mg/kg, ruxolitinib 60 mg/kg, combination of both or vehicle compared to sex- and age-matched untreated MSCV-IRES-GFP_empty vector transplanted BALB/c control mice. Data are represented as mean +/- SEM (***p<0.001, **p<0.01, *p<0.05).
F) CD11b+Gr1- cells in the peripheral blood, bone marrow and spleen at 3 weeks of treatment with C220 at 3 weeks of treatment with C220 at 10 mg/kg, Ruxolitinib 60 mg/kg or combination of both versus vehicle. Data are represented as mean +/- SEM (***p<0.001, **p,0.01, *p<0.05).
G) Total lysed cells in the bone marrow of 3 bones (2 hips, 1 tibia) at 3 weeks of treatment with C220 at 10 mg/kg, Ruxolitinib 60 mg/kg or combination of both versus vehicle. Data are represented as mean +/- SEM (***p<0.001, *p<0.05).
H) Serum cytokine levels (log2) of all 32 cytokines assessed by MCYT MAG- 70K- PX32 (EMD Millipore) at 3 weeks of treatment with C220 at 10 mg/kg, Ruxolitinib 60 mg/kg or combination of both versus vehicle are illustrated. Data are represented as median with 25^{th} and 75^{th} percentiles. p-values between each single and the combination therapy versus vehicle are plotted (***p<0.001, **p<0.01, *p<0.05).

FIGURE S9

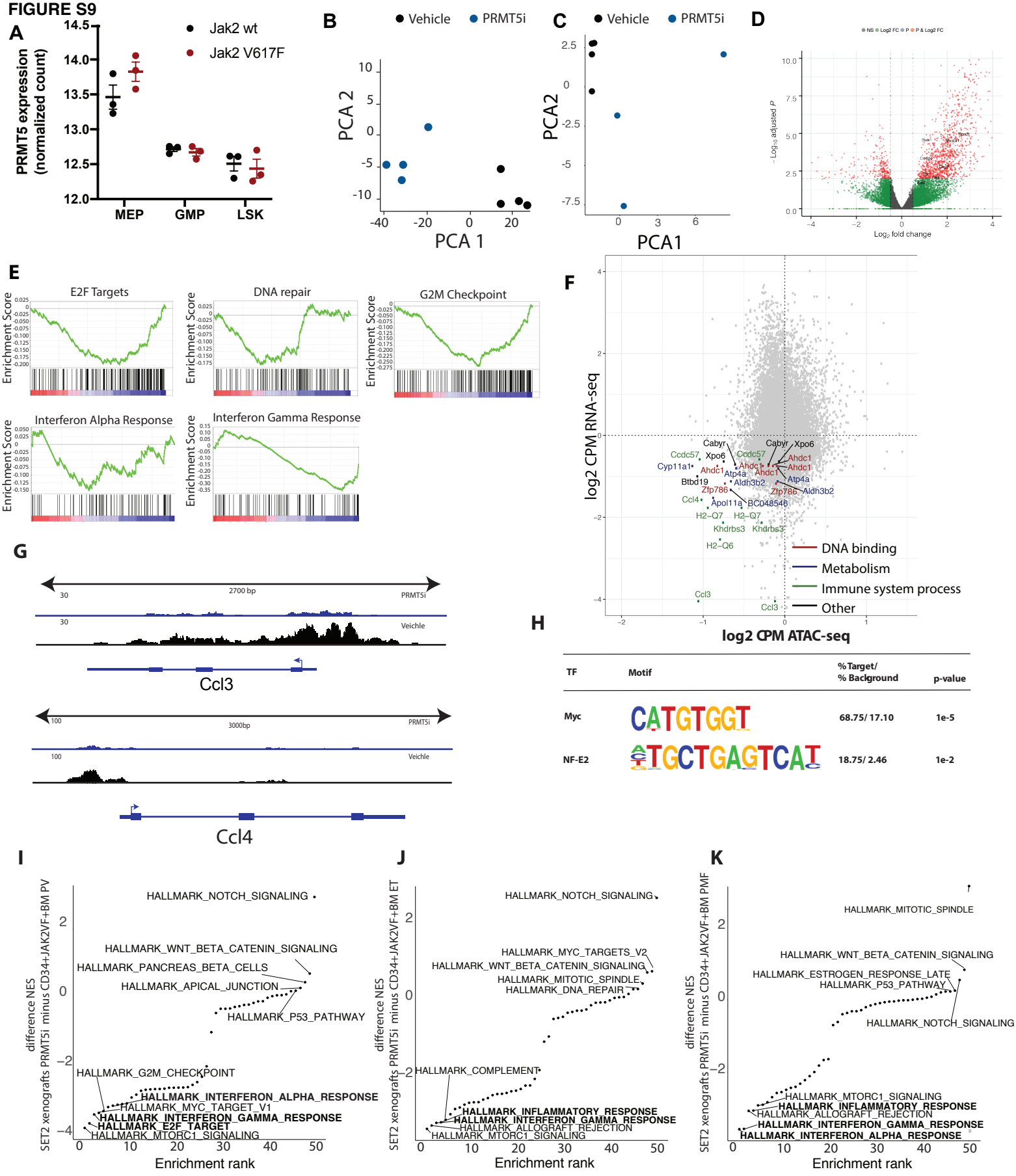

A
PRMT5 expression (normalized count)
Jak2 wt
Jak2 V617F
MEP
GMP
LSK
B
Vehicle
PRMT5i
PCA 1
PCA 2
C
Vehicle
PRMT5i
PCA1
PCA2
D
Log2 fold change
E
E2F Targets
DNA repair
G2M Checkpoint
Interferon Alpha Response
Interferon Gamma Response
Enrichment Score
F
log2 CPM RNA-seq
log2 CPM ATAC-seq
DNA binding
Metabolism
Immune system process
Other
G
2700 bp
PRMT5i
Veichle
Ccl3
3000bp
Ccl4
H
TF
Motif
% Target/ % Background
p-value
Myc
68.75/ 17.10
1e-5
NF-E2
18.75/ 2.46
1e-2
I
J
K
SET2 xenografts PRMT5i minus CD34+JAK2VF+BM PV
SET2 xenografts PRMT5i minus CD34+JAK2VF+BM ET
SET2 xenografts PRMT5i minus CD34+JAK2VF+BM PMF
difference NES
Enrichment rank
HALLMARK_NOTCH_SIGNALING
HALLMARK_WNT_BETA_CATENIN_SIGNALING
HALLMARK_PANCREAS_BETA_CELLS
HALLMARK_APICAL_JUNCTION
HALLMARK_P53_PATHWAY
HALLMARK_G2M_CHECKPOINT
HALLMARK_INTERFERON_ALPHA_RESPONSE
HALLMARK_MYC_TARGET_V1
HALLMARK_INTERFERON_GAMMA_RESPONSE
HALLMARK_E2F_TARGET
HALLMARK_MTORC1_SIGNALING
HALLMARK_MYC_TARGETS_V2
HALLMARK_MITOTIC_SPINDLE
HALLMARK_DNA_REPAIR
HALLMARK_COMPLEMENT
HALLMARK_INFLAMMATORY_RESPONSE
HALLMARK_ALLOGRAFT_REJECTION
HALLMARK_ESTROGEN_RESPONSE_LATE

Figure S9: PRMT5 inhibition reduces expression and accessibility of gene sets associated with Interferon signaling and E2F targets

A) PRMT5 expression in sorted MEP, GMP and LSK populations in the bone marrow of primary JAK2V617F and JAK2-wildtype mice.
B) Principal component analysis (PCA) separating gene expression data according to first 2 components is depicted for SET2 cell derived xenografts after oral treatment with C220 at 15 mg/kg versus vehicle for 2 weeks.
C) Principal component analysis (PCA) separating gene expression data according to first 2 components is depicted for JAK2V617F- positive MEP derived from the JAK2V617F conditional BMT model after treatment with C220 at 15 mg/kg, versus vehicle for 4 weeks.
D) Volcano plot representing differences in gene expression detected by RNA sequencing of tumors derived from the JAK2V617F conditional BMT model after treatment with C220 or vehicle for 4 weeks. The significant events an inclusion level >0.5 log fold change and an FDR corrected p-value <0.0001 are shown in red. Genes with a log fold change <0.5 and a p-value <0.0001 are labeled in blue. Genes with a log fold change >0.5 and a p-value >0.0001 are labeled in green. Genes with a log fold change <0.5 and a p-value >0.0001 are labeled in black.
E) The E2F targets, DNA repair, G2M checkpoint, Interferon alpha and Interferon gamma response signatures are tested for enrichment by GSEA in ATAC-seq data in JAK2V617F-positive MEP derived from the JAK2V617F conditional BMT model after treatment with C220 at 15 mg/kg or vehicle for 4 weeks.
F) Intersection of RNA-seq and ATAC-seq in sorted JAK2V617F-positive MEP after treatment with C220 or vehicle for 4 weeks is plotted as log10(pvalue) *sign of the Log2 fold change of C220 compared to vehicle treatment. Each point represents one ATAC peak.
G) Chromatin accessibility at human locus CCL3 and CCL4 as examples of IFNγ and IL-1 target genes is depicted in SET2 cell derived xenografts after oral treatment with C220 at 15 mg/kg, versus vehicle for 2 weeks.
H) HOMER motif analysis of genes in lower left quadrant of (F) from intersection of RNA-seq and ATAC-seq in JAK2V617F+ sorted MEP after treatment with C220 or vehicle for 4 weeks. Genes that are downregulated and lose accessibility are targets of Myc and NF-E2. p values were calculated using the binominal test.
I) Ranked difference of normalized enrichment score of RNA seq data derived from SET2 cell derived xenografts after oral treatment with C220 at 15 mg/kg versus vehicle for 2 weeks and CD34+ JAK2V617F-positive PV patients(11).
J) Ranked difference of normalized enrichment score of RNA seq data derived from SET2 cell derived xenografts after oral treatment with C220 at 15 mg/kg versus vehicle for 2 weeks and CD34+ JAK2V617F-positive ET patients(11).
K) Ranked difference of normalized enrichment score of RNA seq data derived from SET2 cell derived xenografts after oral treatment with C220 at 15 mg/kg versus vehicle for 2 weeks and CD34+ JAK2V617F-positive PMF patients(12).

FIGURE S10

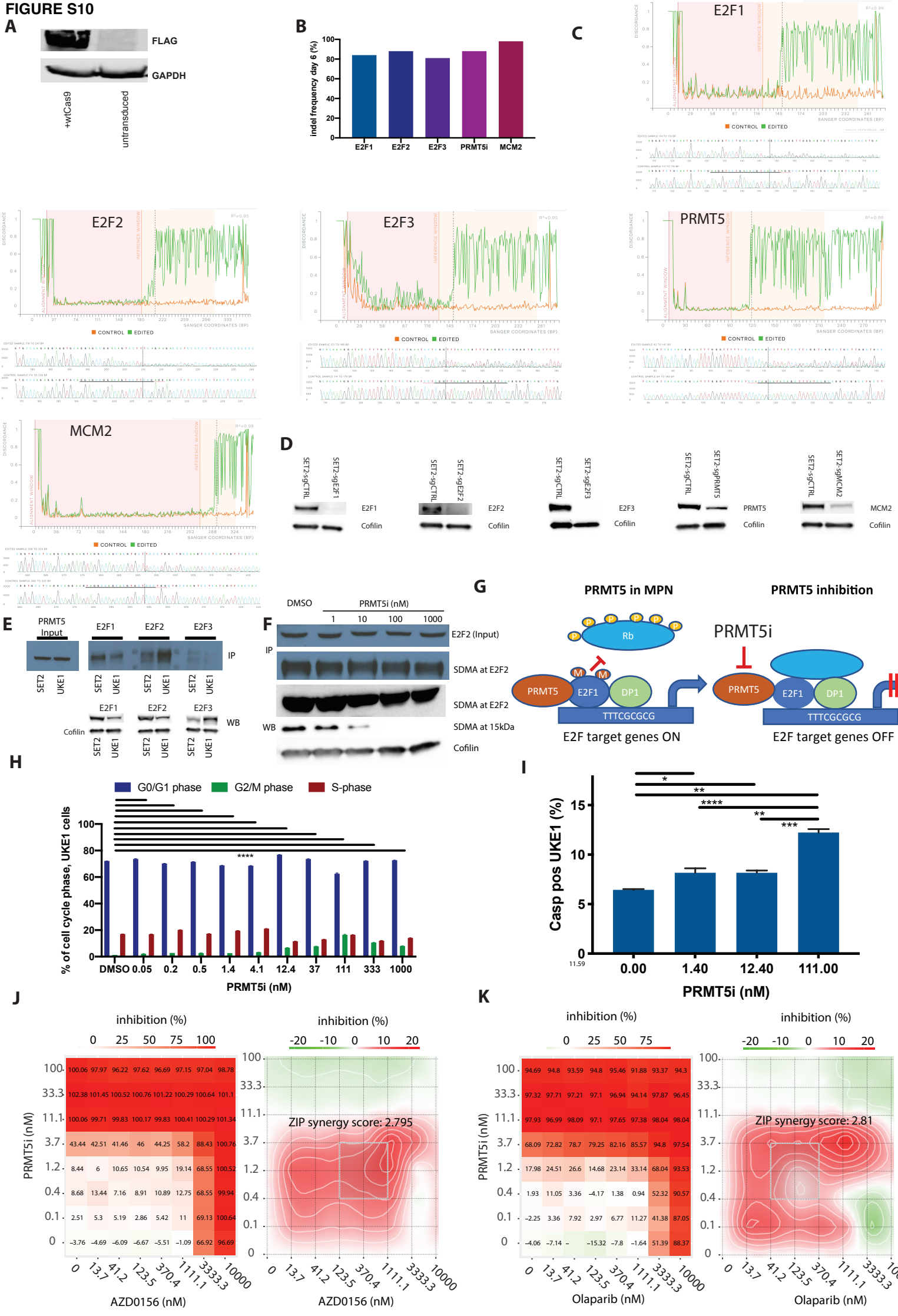

A
FLAG
GAPDH
+wtCas9
untransduced
B
indel frequency day 6 (%)
E2F1
E2F2
E2F3
PRMT5i
MCM2
C
E2F1
E2F2
E2F3
PRMT5
MCM2
CONTROL
EDITED
D
SET2-sgCTRL
SET2-sgE2F1
SET2-sgE2F2
SET2-sgE2F3
SET2-sgPRMT5
SET2-sgMCM2
E2F1
E2F2
E2F3
PRMT5
MCM2
Cofilin
E
PRMT5 Input
E2F1
E2F2
E2F3
IP
WB
Cofilin
SET2
UKE1
F
DMSO
PRMT5i (nM)
1
10
100
1000
E2F2 (Input)
IP
SDMA at E2F2
SDMA at E2F2
WB
SDMA at 15kDa
Cofilin
G
PRMT5 in MPN
PRMT5 inhibition
Rb
PRMT5
E2F1
DP1
PRMT5i
TTTCGCGCG
E2F target genes ON
E2F target genes OFF
H
G0/G1 phase
G2/M phase
S-phase
% of cell cycle phase, UKE1 cells
DMSO
0.05
0.2
0.5
1.4
4.1
12.4
37
111
333
1000
PRMT5i (nM)
I
Casp pos UKE1 (%)
0.00
1.40
12.40
111.00
PRMT5i (nM)
J
inhibition (%)
PRMT5i (nM)
AZD0156 (nM)
ZIP synergy score: 2.795
K
inhibition (%)
PRMT5i (nM)
Olaparib (nM)
ZIP synergy score: 2.81

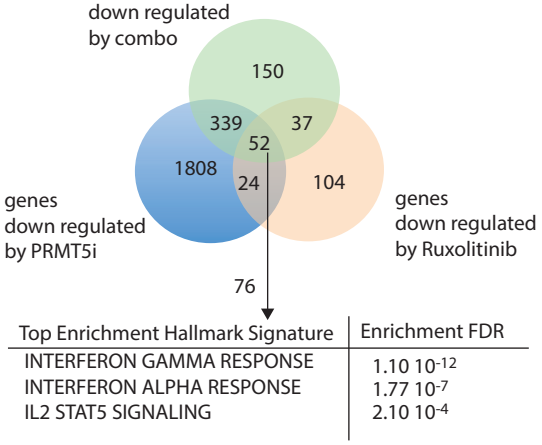
Figure S10: PRMT5 inhibition reduces expression and accessibility of gene sets associated with Interferon signaling and E2F targets

A) Expression of FLAG in SET2 cells transduced with FLAG tagged Cas9wt (pLentiCas9-blast) or untransduced SET2 cells.
B) Indel frequency measured at day6 after transduction of stable expressing SET2 Cas9 cells with pU6-sgRNA-EF1α-puro-T2A-BFP vectors containing sgRNA for E2F1, E2F2, E2F3, PRMT5 or MCM2 (used as a positive control), respectively, in BFP+ sorted SET2 cells.
C) Discordance plots detailing the level of alignment per base between the wild type (control) and the edited sample in the inference window are depicted for CRISPR edited cells lines used in competition assay (E2F1, E2F2, E2F3, PRMT5 and MCM2).
D) Western blot confirmation of target knockdown in cell lines used in the competition assay[1].
E) Western blot assessment of E2F1, E2F2 and E2F3 expression in total protein lysate and after immunoprecipitation of PRMT5 in SET2 and UKE cells is illustrated[1].
F) Western blot assessment of expression of symmetrically dimethylated E2F2 (SDMA at E2F2) in total protein lysate and after immunoprecipitation of E2F2 in SET2 cells treated with different doses of inhibitor or DMSO control for 6 days is illustrated[1].
G) Proposed model for methylation of E2F1 by PRMT5 in MPN cells. PRMT5 symmetrically demethylates E2F1 in MPN cells which prevents binding of Rb protein and switches E2F target genes on leading to proliferation. Vice versa, inhibition of E2F1 methylation by PRMT5 inhibitor facilitates Rb binding and turns E2F target genes off.
H) Cell cycle proportion is determined by flow cytometry of UKE1 cells treated with different doses of inhibitor or DMSO control for 6 days using the FITC BRDU Flow Kit (BD). Data are indicated as mean ± SEM (****p<0.0001, for comparison of % of cells in G2/M phase)
I) Induction of apoptosis in UKE1 cells treated with increasing doses of C220 or DMSO control for 6 days is measured as proportion of caspase-3 positive cells in flow cytometry and plotted as bar graphs. Data are indicated as mean ± SEM (*p<0.05,**p<0.01,***p<0.001, ****, p<0.0001).
J) Visualization of the dose-response matrix and the plots of phenotypic responses for the single C220 and AZD0156 treatment in SET2 cells treated for 9 days. Raw dose-response matrix data derived from proliferation synergy assay for the combination of PRMT5 inhibition by C220 and ATM inhibition by AZD0156 in SET2 cells is visualized as a heatmap.
K) Visualization of the dose-response matrix and the plots of phenotypic responses for the single C220 and Olaparib treatment in SET2 cells treated for 9 days. Raw dose-response matrix data derived from proliferation synergy assay for the combination of PRMT5 inhibition by C220 and PARP inhibition by Olaparib in SET2 cells is visualized as a heatmap.

[1]Note: For WB and IP analyses, all images that appear in blue scale were acquired with conventional film and scanned, all images that appear in gray and white scale were electronically acquired.

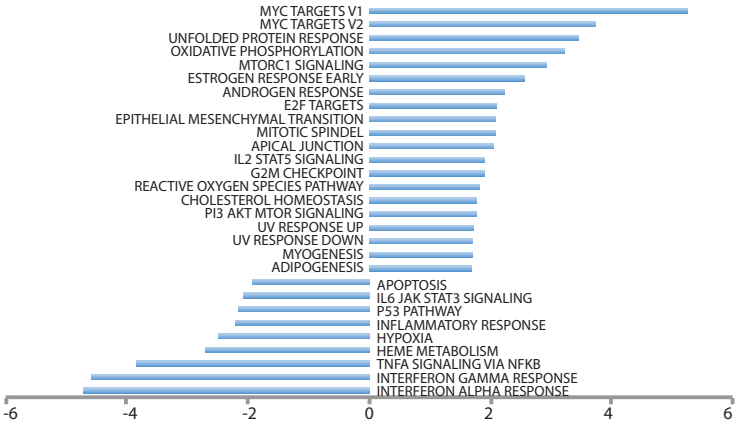
FIGURE S11

A

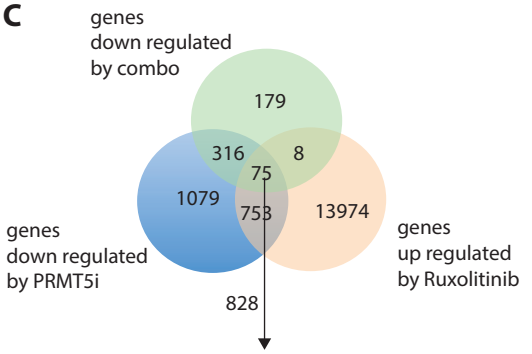


Top Enrichment Hallmark Signature	Enrichment FDR
INTERFERON GAMMA RESPONSE	$1.10\ 10^{-12}$
INTERFERON ALPHA RESPONSE	$1.77\ 10^{-7}$
IL2 STAT5 SIGNALING	$2.10\ 10^{-4}$

B



C



Top Enrichment Hallmark Signature	Enrichment FDR
E2F TARGETS	$1.12\ 10^{-18}$
MTORC1 SIGNALING	$1.12\ 10^{-14}$
MYC TARGETS V1	$1.34\ 10^{-9}$

D

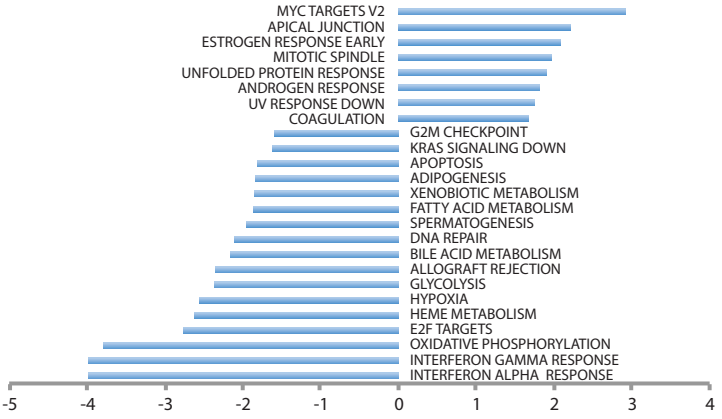


Figure S11: Differential gene expression in SET2 xenografts induced by treatment with C220, Ruxolitinib or combination of both

A) Venn diagram showing the overlap between downregulated genes in SET2 xenografts after 2 weeks of treatment with C220, ruxolitinib, or combination versus vehicle. The cutoffs were fold change >0.05 with a false discovery rate (FDR) adjusted p-value of <0.01.
B) HALLMARK gene set enrichment analysis of genes affected by Ruxolitinib inhibition in SET2 xenografts treated for 2 weeks. NES: normalized enrichment score.
C) Venn diagram showing the overlap between genes downregulated by C200 or combination treatment and genes upregulated by ruxolitinib in SET2 xenografts after 2 weeks of treatment with C220, ruxolitinib, or combination versus vehicle.. The cutoffs were fold change >0.05 with a false discovery rate (FDR) adjusted p-value of <0.01.
D) HALLMARK gene set enrichment analysis of genes affected by dual C220/Ruxolitinib inhibition in SET2 xenografts treated for 2 weeks. NES: normalized enrichment score.